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Mechanism of Electromethanogenic Reduction of CO_2 by a Thermophilic Methanogen

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Abstract

To establish a biotechnological system to convert CO_2 into methane, we are trying to develop a new CO_2 bio-conversion technology based on "electromethanogenesis", a new bio-electrolysis reaction using microbially-catalyzed electrode. In this study, we characterized bio-electrochemical properties of electromethanogenic reaction by *Methanothermobacter thermautotrophicus* strain ΔH, a thermophilic methanogen. The methanogen can electromethanogenically produce methane without exogenously-supplied hydrogen. In the reaction, the methanogen utilized molecular hydrogen, which was evolved by the abiotic electrochemical reaction, for the hydrogenotrophic methanogenesis. The current-to-methane conversion efficiency was 20% and the hydrogen evolution reaction was the rate-limiting step of the reaction.

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Keywords : methanogen ; electromethanogenesis ; methane production ; molecular hydrogen ; bio-electrolysis reaction

1. Introduction

In the IEA BLUE Map scenario, to reduce GHG emissions by 50% by 2050, it is estimated that 100 Carbon dioxide Capture and Storage (CCS) projects need to be deployed by 2020 and over 3000 projects by 2050 [1]. However, the deployment of CCS is currently limited to only eight fully-integrated

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operations (Sleipner, Snohvit, In Salah, Weyburn, Shute Creek, Val Verde, Enid Fertilizer and Century projects [2]) and largely affected by legal and regulatory aspects, public acceptance and financial issues.

In an effort to create an additional incentive for the CCS deployment, the long-term aim of this research is to establish a biotechnological system to microbiologically convert geologically-stored CO_2 into methane, an energy resource. To develop a means for the conversion, we focus on technological application of a bio-electrochemical reaction called “electromethanogenesis”.

It has been well known that a group of methanogens (collectively called hydrogenotrophic methanogens) reduces CO_2 into methane by using molecular hydrogen (hydrogenotrophic methanogenesis: Eq. 1) [3].

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (1)$$

Methanogens have been almost ubiquitously detected in anoxic environments including wetlands, marine sediments, animal gastroenterology systems and subsurface formations (such as oil/gas reservoirs) [3] [4]. Moreover, many of natural-gas fields are thought to be generated by the metabolic activity of subsurface methanogens. Thus, it seems feasible to recruit the reservoir-endogenous methanogens for the methanogenic conversion of geologically-stored CO_2. However, an effective means to supply reducing power source(s) remains to be established (Obviously, injecting molecular hydrogen into the reservoir is hardly practical).

Electromethanogenesis is a bio-electrochemical reaction using microbially-catalyzed electrode (biocathode) [5]. On the cathode surface, microbial consortia utilize, instead of molecular hydrogen, proton and electrical current (electrons) as the reducing power source to reduce CO_2 as in Eq. 2.

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \quad (2)$$

Electromethanogenesis provides, as a core reaction of new CO_2-conversion/utilization technology, an interesting possibility. As it has been reported that the conversion efficiency of electrons (current) consumed at the cathode into methane was as high as 96% [6], it allows us to store electrical energy in a stable form, methane. Particularly, by applying electromethanogenesis to convert geologically-stored CO_2, the CO_2-storage reservoir can serve as a large-scale energy-storage tank. Such technological system can be useful for storage of intermittent electrical energy from renewable power sources (such as solar and wind).

However, as electromethanogenesis has been found recently [6, 7], our knowledge on the reaction is still limited. Particularly, since previous studies have exclusively focused on mesophilic systems, electromethanogenesis under thermophilic condition (i.e. condition within subsurface reservoir) has never been examined. Moreover, although the electromethanogenic consortium contained a hydrogenotrophic methanogen, direct role of the methanogen in electromethanogenesis is yet to be elucidated. Toward technological application of the bio-electrochemical system in CO_2 geological reservoir, bio-electrochamical properties and the pathway of electromethanogenic reaction by pure culture of a thermopilic methanogen were analyzed.

2. Materials and Methods

2.1. Microbial material

Methanothermobacter thermautotrophicus strain ΔH was used as a model species of thermophilic methanogens, as the methanogen has been almost ubiquitously detected in subsurface environments [8][9].

2.2. Pre-cultivation of the methanogen

For cultivation of *M. thermautotrophicus* strain ΔH, anaerobically-prepared *Methanobacterium* media [NITE medium #1067; containing 0.136 g KH_2PO_4, 0.54 g NH_4Cl, 0.2 g $MgCl_2 \cdot 6H_2O$, 0.147 g $CaCl_2 \cdot 2H_2O$, 2.5 g $NaHCO_3$, 0.5 g cysteine · HCl, 0.5 g $Na_2S \cdot 9H_2O$, 0.8 g sodium acetate, 0.2 g yeast extract, 1 mg resazurin, 6.4 mg EDTA, 62 mg $MgSO_4 \cdot 7H_2O$, 5.5 mg $MnSO_4 \cdot H_2O$, 10 mg NaCl, 1 mg $FeSO_4 \cdot 7H_2O$, 1 mg $Co(NO_3)_2 \cdot 6H_2O$, 1 m g $CaCl_2$, 1 mg $ZnSO_4 \cdot 7H_2O$, 0.1 mg $CuSO_4 \cdot 5H_2O$, 0.1 mg $AlK(SO_4)_2$, 0.1 mg H_3BO_3, 0.1 mg $Na_2MoO_4 \cdot 2H_2O$, 0.01 mg Na_2SeO_3, 0.1 mg $Na_2WO_4 \cdot 2H_2O$, 20 μg biotin, 20 μg folic acid, 100 μg pyridoxine-HCl, 50 μg thiamine-HCl, 50 μg riboflavin, 50 μg nicotinic acid, 50 μg Ca-pantothenate, 10 μg *p*-aminobenzoic acid, 0.1 μg vitamin B_{12} (per liter)] was used .

50 ml of *Methanobacterium* media was added into vial bottles (200 ml vol., Maruemu, Osaka, Japan) under anaerobic condition. After the strain was inoculated into the medium, the vials were sealed by butyl rubber stoppers and aluminum seals. The headspaces of bottles were exchanged with H_2/CO_2 (80/20), then the strain was cultured in an air-bath incubator (Taitech, Aichi, Japan) at 65℃ with shaking at 180 rpm for 16 hours.

100 ml of the preculture was added into small vial bottles (10 ml volume, Maruemu), sealed by butyl rubber stoppers and aluminum seals under anaerobic condition. Cells were harvested by centrifugation at 2500 rpm for 30 min by a refrigerated centrifuge (CAX-370, Angle Rotor CA-9: TOMY Co., Tokyo) and resuspended in fresh *Methanobacterium* media. The cell density was adjusted to $^{1cm}OD_{600}$ of 0.2, measured by a spectral photometer (6300 pro: GE healthcare, Tokyo, Japan).

2.3. Electrochemical reactor construction

2.3.1. Electrode preparation

The anode electrodes were made of plain carbon paper (3 cm^2: Tsukuba Materials Information Laboratory, Tsukuba, Japan). The cathode electrodes were made of carbon paper (3 cm^2) coated with a carbon layer [2.5 mg/cm^2 Vulcan XC-72R (Cabot, Billerica, MA, USA), using Nafion (Sigma-Aldrich, St. Louis, MO, USA) as binder] on one side. Titanium wires (0.5 mm in diameter: Alfa Aesar, Ward Hill, MA, USA) were attached to the electrodes by using conductive epoxy CW2400 (Chemtronics, Kennesaw, GA, USA) and used to connect the electrodes to the circuit.

2.3.2. Reactor assembling

Single-chamber electrochemical reactors were constructed using 10 ml vial bottles (Fig. 1). A nylon filter (NY6H: Millipore, Billerica, MA, USA) was inserted between the cathode and the anode to prevent direct contact between the electrodes. 8 ml of *M. thermautotrophicus* cell suspension was inoculated into each reactor. The reactors were sealed with butyl-rubber stoppers and incubated anaerobically [N_2-CO_2 (80:20, v/v)] at 60°C without agitation. Constant voltage was applied to the reactors with the positive pole connected to the anode and the negative pole to the cathode using a DC power supply (Array 3645A: Array Electronics, Nanjing, China).

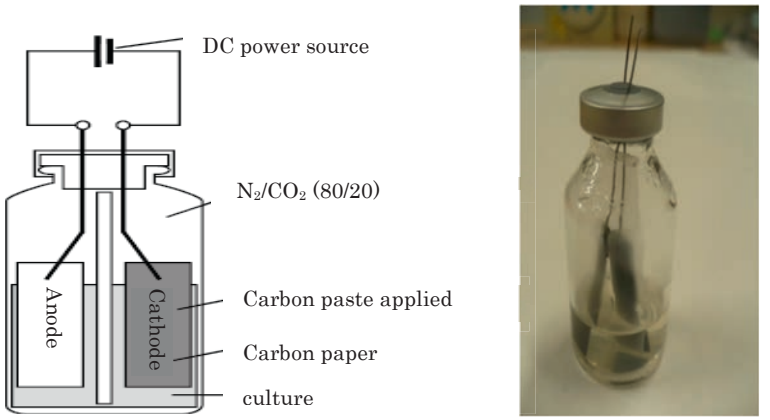

Fig. 1 A schematic drawing (left) and picture (right) of the electrochemical reactor cell

2.4. Analytical methods

2.4.1. Gas concentration

The concentrations of methane and molecular hydrogen in the reactor headspace were measured at appropriate intervals using a GC-2014 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a Shincarbon ST column (6 m × 3 mm ID, Shimadzu).

2.4.2. Current generation and methane conversion rate

To measure the current produced in the reactor, the voltage across the fixed external resistance (1 Ω) was monitored by using a multimeter (34970A, Agilent Technologies, Santa Clara, CA, USA). The conversion rate from current to methane was calculated by the following equation [10]. F is Faraday constant (96485 C mol^{-1}) and I is the current (A).

$$methane\ recovery\ rate\ (\%) = \frac{the\ amount\ of\ electron\ incorporated\ into\ methane}{the\ amount\ of\ electron\ comsumed\ in\ the\ circuit} \times 100$$

$$= \frac{the\ amount\ of\ methane\ production\ (mol) \times 8}{\int_{t=0}^{t} I / 2F} \times 100 \qquad (3)$$

2.4.3. Linear sweep voltammetry (LSV)

To examine the current densities in the reactor, LSV was employed by using a potentiostat (HSV-110, Hokuto Denko, Tokyo, Japan) with a standard three-electrodes system. The cathode, the anode and an Ag/AgCl reference electrode inserted into the reactor were acted as the working electrode, counter electrode and reference electrode, respectively. LSV was conducted in the potential range from -0.1 to -0.8 V (vs. SHE) at a low scan rate of 1.0 mV/s.

3. Result and Discussion

3.1. Applied-voltage-dependent methane production by M. thermautotrophicus strain ΔH

To examine whether *M. thermautotrophicus* strain ΔH has electromethanogenic activity, the pure culture of *M. thermautotrophicus* strain ΔH was incubated with an applied voltage of 1.0 V (1 V in Fig. 2) in the electrochemical reactors. No molecular hydrogen was exogenously added to the reactors. Active methane accumulation was observed until six hour post initiation of the incubation (hpi) but then slowed down and almost ceased later than 24 hpi (Fig. 2). The maximum methane production rate was 87.9 mmol day^{-1} m^{-2} (the cathode surface area), which was almost eight times higher than that of the pure culture of a mesophilic methanogen *M. palustre* strain ATCCBAA-1077 (10.8 mmol day^{-1} m^{-2}) [5]. To further examine the effect of applied voltage on electromethanogenesis by *M. thermautotrophicus* strain ΔH, a range of voltages (from 0.5 to 1.5 V) were applied to the reactors. The methane accumulation depended on the level of applied voltage (i.e. more methane was produced with higher level of applied voltage) (data not shown). No significant methane production was observed in the reactors without applied voltage (0 V in Fig. 2). Thus, this result indicated that *M. thermautotrophicus* strain ΔH had electromethanogenic activity and, however, the activity was attenuated in the later stage of incubation.

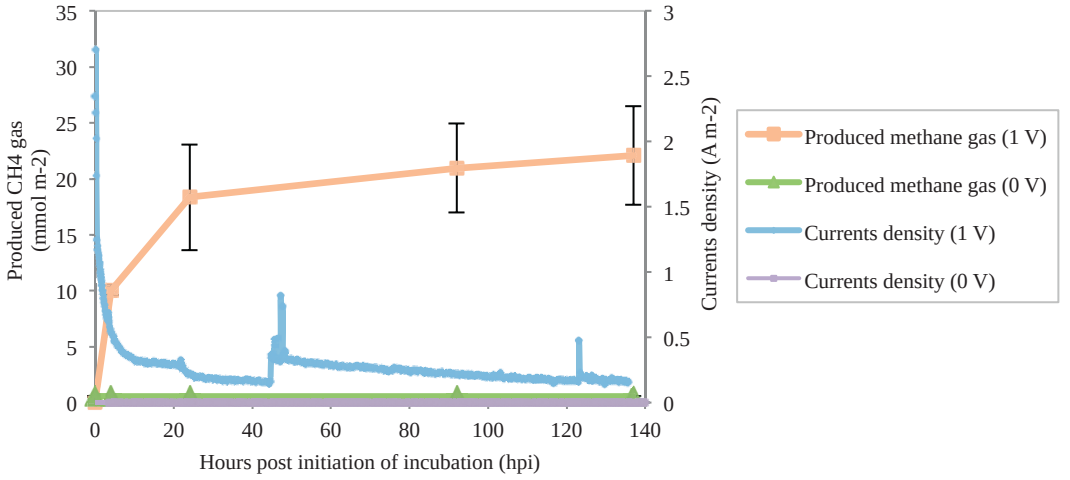

Fig. 2 Methane and current production in the electrochemical reactors inoculated with *M. thermautotrophicus* strain ΔH with an applied voltage of 1.0 V (1 V) or without applied voltage (0 V)

Current generation in the circuit was nearly proportional to the methane production activity and also depended on the applied voltage (Fig. 2), suggesting that the current produced in the circuit was consumed for the methane production. However, the current-to-methane conversion rate was *ca.* 19%, which was significantly lower than that (96%) of the mesophilic microbial consortium [6].

3.2. Electrochemical property of the electromethanogenic reaction by M. thermautotrophicus strain ΔH

The electron consumption possible in the cathode inoculated with *M. thermautotrophicus* strain ΔH, was examined by LSV analysis. Resulting voltammograms of the cathodes with the methanogen were almost identical to those of the non-inoculated control (Fig. 3). In other words, electron consumption at the cathode did not depend on the presence of the methanogen. Thus, the LSV result suggested that,

although the methane production required presence of the methanogen and voltage application, the reaction responsible for electron consumption at the cathode was independent of the methanogen. This was also in contrast to the previous report with the mesophilic microbial consortium, in which the electron consumption largely depended on the presence of the cathodic consortium [6].

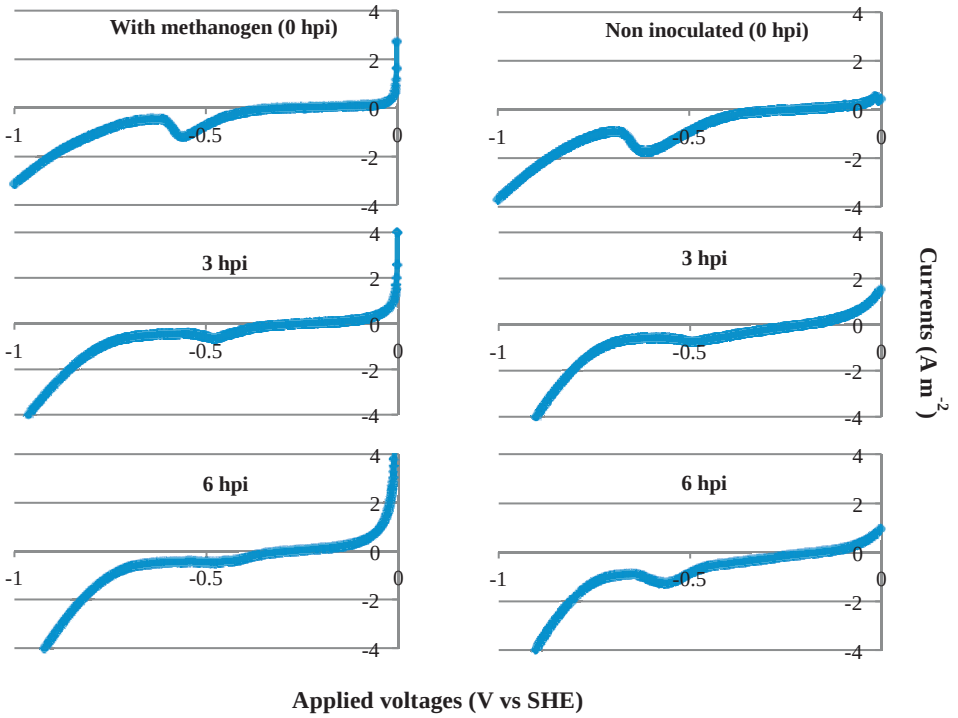

Fig. 3 Linear sweep voltammograms of the cathode inoculated with *M. thermautotrophicus* strain ΔH (left panels) and a non-inoculated cathode (right panels) at 0, 3 and 6 hours post initiation of the voltage (1.0 V) applied incubation (hpi).

3.3. M. thermautotrophicus strain ΔH utilized molecular hydrogen evolved by abiotic electrochemical reaction in the reactors

To understand nature of the methanogen-independent reaction on the cathode, the electrochemical reactors were operated without the methanogen. No methane but molecular hydrogen was produced in an applied-voltage-dependent manner (Fig. 4). Thus it was suggested that, in the absence of the methanogen, molecular hydrogen was electrochemically evolved on the cathode as in Eq. 4.

$$2H^+ + 2e^- \rightarrow H_2 \quad (4)$$

Moreover, the amounts of molecular hydrogen produced were equivalent to the methane produced in the presence of the methanogen. Thus, these observations suggested that, in the electromethanogenic reaction by *M. thermautotrophicus* strain ΔH (Fig. 2), the methanogen utilized molecular hydrogen, which was evolved by the abiotic electrochemical reaction (as in Eq. 4), for the hydrogenotrophic methanogenesis (as in Eq. 1). The conversion efficiency from currents to molecular hydrogen was *ca.*

20%. On the other hand, the conversion efficiency from evolved hydrogen to methane was estimated to be *ca.* 100% (by comparing the amount of the produced methane in the presence of *M. thermautotrophicus* strain ΔH and those of the molecular hydrogen in the absence of the methanogen). Thus, this result indicated that the hydrogen evolution (electron consumption) reaction at the cathode was the rate-limiting step of the electromethanogenesis by the *M. thermautotrophicus* pure culture.

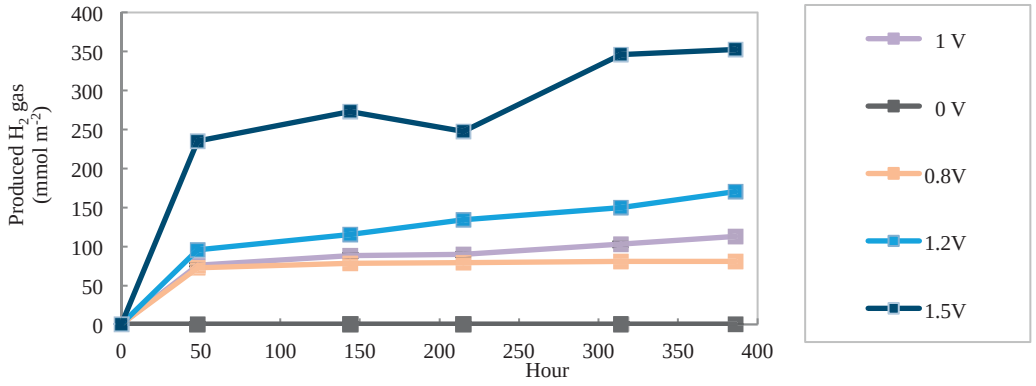

Fig. 4 Hydrogen production in the non-inoculated electrochemical reactors with a range of applied voltage (0 – 1.5 V).

4. Conclusion

In this study, we examined the mechanism of the electromethanogenic reaction by a pure culture of *M. thermautotrophicus* strain ΔH. The methanogen can electromethanogenically produce methane without exogenously-supplied hydrogen. In the reaction, the methanogen utilized molecular hydrogen, which was evolved by the abiotic electrochemical reaction, for the hydrogenotrophic methanogenesis. The current-to-methane conversion efficiency was 20% and the hydrogen evolution reaction was the rate-limiting step of the reaction. In previous study, it has been shown that, in the electromethanogenic reaction by a mesophilic microbial consortium, the current-to-methane conversion efficiency was as high as 96%. Our result suggested that, in such electromethanogenic consortia, microorganism(s) other than methanogen play a role as mediator, which catalyzes electron transfer from the cathode to methanogen. Thus, to utilize electromethanogenesis as a technology to convert geologically-stored CO_2 into methane, thermophilic mediator microorganism(s) with such mediator activity will be required.

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References

[1] IEA, International Energy Agency. Technology Roadmap Carbon Capture and Storage, IEA/OECD. Paris: 2009.

[2] GCCSI, Global CCS Institute. The Global Status of CCS. 2011, Chapter 2, 8-32.

[3] Nagina TN, Shestakova NM, Grigor'yan AA, Mikhailova EM, Tourova TP, Poltaraus AB, *et al.* Phylogenetic diversity and activity of anaerobic microorganisms of high-temperature horizons of the Dagang oil field. *Microbiology.*2006;**75**:55-65.

[4] Mochimaru H, Yoshioka H, Tamaki H, Nakamura K, Kaneko N, Sakata S, *et al.* Microbial diversity and methanogenic potential in a high temperature natural gas field in Japan. *Extremophiles*. 2007;**11**:453-361.

[5] Demirel B, Scherer P. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane a review. *Rev Environ Sci Biotechnol* 2008;**7**:173-190.

[6] Cheng S, Xing D, Call FD, Logan EB. Direct Biological Conversion of Electrical Current into Methane by Electromethanogenesis. *Environ. Sci. Technol.* 2009;**43**:3953-3958.

[7] Villano M, Aulenta F, Ciucci C, Ferri T, Giuliano A, Majone M. Bioelectrochemical reduction of CO_2 to CH_4 via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture. *Bioresour Technol* 2010;**101**:3085-90.

[8] Kobayashi H, Endo K, Sakata S, Mayumi D, Kawaguchi H, Ikarashi M. *et al.* Phylogenetic diversity of microbial communities associated with the crude-oil, large-insoluble-particle and formation-water components of the reservoir fluid from a non-flooded high-temperature petroleum reservoir. *J Biosci Bioeng.* 2012;**113(2)**:84-7.

[9] Kobayashi H, Kawaguchi H, Endo K, Mayumi D, Sakata S, Ikarashi M. *et al.* Analysis of methane production by microorganisms indigenous to a depleted oil reservoir for application in Microbial Enhanced Oil Recovery. *J Biosci Bioeng.* 2012;**113(1)**: 84-7.

[10] Logan EB. Power generation. In: *MICROBIAL FUEL CELLS*. John Wiley & Sons, Inc. U.S.A., 2008, p. 44-60.